Sodium, Calcium and Potassium Channels

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Secondary article

Article Contents

Sodium, calcium and potassium channels form the basis for the electrical excitability of various cell types. They are membrane proteins which harbour voltage-gated pores providing selective permeation pathways for Na^+, Ca^{2+} and K^+ ions, respectively.

Introduction

Voltage-gated sodium (Na^+), calcium (Ca^{2+}), and potassium (K^+) channels form the functional basis for the generation and propagation of action potentials in excitable cells. Owing to their different ionic selectivities, i.e. their ability to selectively conduct certain ion species, the fine-tuned interplay of the opening and closing of these ion pores gives rise to complex electrical signals. While the opening of sodium and calcium channels results in the depolarization of cells (as both Na^+ and Ca^{2+} follow their electrochemical gradient and flow into the cell), the opening of potassium channels hyperpolarizes the cells as K^+ ions leave the cell at voltages more positive than about -80 mV. Therefore, the major purpose of sodium and calcium channels is the initiation and prolongation of action potentials. In some cases this results in an increase in intracellular Ca^{2+} concentration that, for instance, leads to the contraction of heart muscle cells. Termination of action potentials is mediated by the inactivation of sodium and calcium channels as well as by the activation of voltage-gated potassium channels. The overall excitability of cells is largely determined by those potassium channels that are open at typical resting potentials. Thus, while the voltage-dependent gating of sodium and calcium channels is rather uniform – i.e., rapid opening upon depolarization and (in some cases) subsequent inactivation – potassium channels are much more diverse in their functional properties. They range from rapidly activating and inactivating channels (A type channels), through those that activate upon depolarization without significant inactivation (delayed rectifier channels), to those that close even upon depolarization but which conduct K^+ in the range of typical resting potentials (inward rectifying channels) (see **Figure 2**).

Although these ion channels are characterized by quite distinct ion selectivities, diverse voltage-dependent gating kinetics and very specific pharmacological properties, they share many structural features that became apparent upon molecular cloning of several genes coding for sodium, calcium and potassium channel proteins. The channel-forming protein complex has a real or pseudo 4-fold symmetry: While four α subunits of potassium channels have to assemble in order to form an ion channel (see **Figure 2**), sodium and calcium channel α subunits harbour this 4-fold symmetry in one polypeptide which shows four homologous domains (**Figure 1**). In all cases the amino- and carboxy-terminal ends face the cytosol. The largest subfamily of potassium channel α subunits exhibits six putative transmembrane segments (6TM: S1–S6, see Potassium Channels, below); sodium and calcium channels have 4*6 such segments (4*6TM). Common to most channels of this topological organization is their activation (opening) upon membrane depolarization. The sensing of the transmembrane electric field is mediated by the fourth segment (S4), which is a putative transmembrane segment with a high density of positively charged amino acid residues. Inward rectifying channels lack this activation mechanism and they are composed only of α subunits with two transmembrane segments (2TM: M1–M2, see Potassium Channels, below). Despite this strong difference, they share a similar pore-forming structural motif with the other voltage-gated channels; this motif (commonly called P-region or H5-loop) is the connecting loop between S5 and S6 or M1 and M2, respectively (**Figure 2**).

Based on structural rather than on functional similarity to voltage-gated channels, ion channels that are gated by intracellular Ca^{2+} (see Ca^{2+}-Dependent Potassium Channels, below) and cyclic nucleotides (see Cyclic Nucleotide-gated Channels, below) are also considered here.

Voltage-gated Sodium and Calcium Channels

Sodium and calcium channels are the excitatory elements in many electrically active cells. They open a permeation pathway for Na^+ and Ca^{2+} ions, respectively, upon depolarization of the cell membrane. As the pore-forming protein subunits of these channels belong to the same protein superfamily, they share many structural and functional similarities.

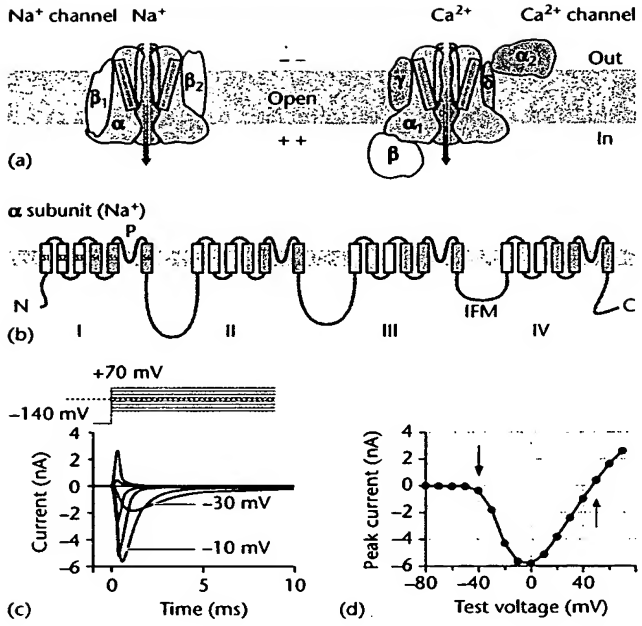

Figure 1 Functional and structural properties of voltage-gated sodium and calcium channels. (a) Subunit composition of voltage-gated sodium and calcium channels. (b) Putative organization of the α subunit of sodium channels in the membrane. The boxes indicate protein segments that are highly likely to span the membrane. The S4 segment (red) contains a high density of positively charged residues and serves as a voltage sensor. The green loop (P-region) is the major determinant for the ion permeation pathway. The IFM motif (amino acids Ile-Phe-Met) in the cytosolic linker between repeats III and IV is important for the rapid channel inactivation. (c) Voltage-clamp current recordings from mammalian host cells transfected with the μl sodium channel gene from rat skeletal muscle. The current traces are responses to the indicated steps in membrane potential. (d) Peak current versus test voltage from the experiment shown in (c). Note the activation threshold of sodium channels at around − 40 mV and the reversal potential for Na^+ ions close to +50 mV (arrows).

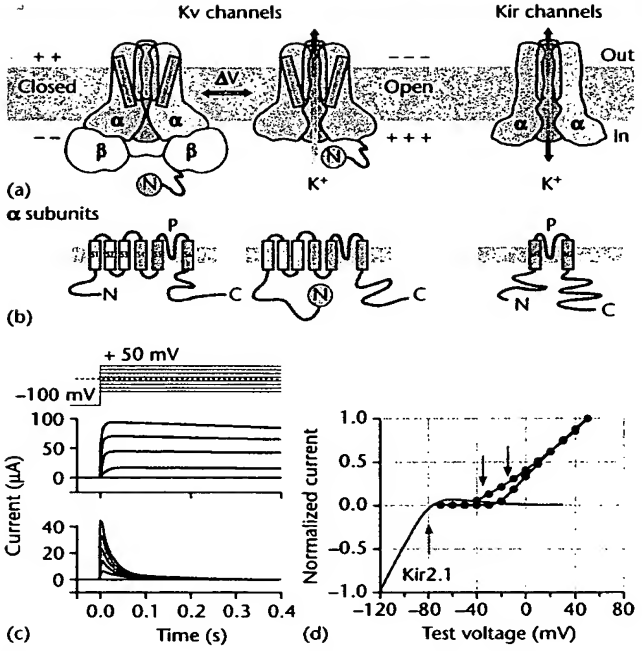

Figure 2 Functional and structural properties of voltage-gated and inward rectifying potassium channels. (a) Subunit composition of voltage-gated (Kv) and inward rectifying (Kir) potassium channels. The channel-forming pores are composed of four α subunits (grey). α Subunits of Kv channels have a voltage-sensing S4 transmembrane segment that undergoes conformation changes upon changes in the transmembrane electric field inducing closed (left) and open (right) states of the channel pore. Some species of Kv channels associate with cytosolic β subunits (left). (b) Putative organization of the α subunits of potassium channels in the membrane. Kir channels lack segments S1–S4. (c) Voltage-clamp current recordings from *Xenopus laevis* oocytes that were injected with mRNA coding for the delayed rectifier channel hKv1.5 (black) and the transient A type potassium channel rKv1.4 (red). (d) The peak currents of the experiments shown in (c) are plotted as a function of test voltage (circles). Also included in this current–voltage plot is a current trace recorded from oocytes expressing the inward rectifier channel mKir2.1 (green). The black and red arrows indicate the activation thresholds for the Kv channels; the green arrow indicates the reversal potential for K channels.

Sodium channels

Voltage-gated sodium channels initiate fast action potentials. When the resting membrane voltage exceeds a critical value (activation threshold) of about − 40 to − 30 mV (**Figure 1d**), these channels open within less than 1 ms, leading to an inward flux of Na^+ ions which depolarizes the cell towards the reversal potential for Na^+ (about + 40 to + 50 mV, **Figure 1d**). Activation is followed by spontaneous inactivation of sodium channels; i.e. the channels close during a depolarization (**Figure 1c**). This inactivation proceeds within a few milliseconds and constitutes the first step in the termination of action potentials. Upon hyperpolarization of the cells, a certain lag time is required for the sodium channels to recover from inactivation. During that refractory period (~ 10–100 ms), sodium channels cannot be activated again and, hence, no new action potential can be initiated (Hodgkin and Huxley, 1952). Activation of sodium channels is mainly governed by the S4 structures of the four homologous repeats, while channel inactivation is mainly determined by the cytosolic polypeptide loop connecting the homologous repeats III and IV of the α subunit (Stühmer *et al.*, 1989; West *et al.*, 1992).

Sodium channels have a typical single-channel conductance of 10–20 pS (1 picosiemens = 10^{-12} ampere/volt) under physiological conditions. They display a clear selectivity of small (Na^+ and Li^+) over larger (K^+, Cs^+, Rb^+) monovalent cations (permeability ratio for Na^+ over K^+ is typically 10–20); they are hardly permeable for divalent cations (Ca^{2+}) and do not conduct anions (e.g. Cl^-). From the permeability of organic cations, a limiting

dimension of the ion pore of about 0.3 × 0.5 nm can be estimated. The ion selectivity of the channel is mainly determined by single residues in the P-region since, for example, the replacement of the lysine at position 1422 in rat brain II sodium channel by the negatively charged glutamate results in Ca^{2+}-permeable channels without Na^+/K^+ selectivity (Heinemann *et al.*, 1992).

Sodium channels are targets for various pharmacological agents and peptide toxins. These bind to receptor sites, causing channel block or modification of the gating properties. Tetrodotoxin (TTX) and saxitoxin are the best-known toxins that inhibit ion conductance by binding at the extracellular side of the pore. Local anaesthetics induce use-dependent channel inhibition. Lipid-soluble toxins like aconitine, veratridin, and batrachotoxin as well as the pyrethroid insecticides and DDT shift the voltage dependence of activation and slow down inactivation. α-Scorpion toxins and sea-anemone toxins also inhibit inactivation, leading to hyperexcitability of the target cells. Intracellular endopeptidases such as pronase remove inactivation presumably by attacking the inactivating polypeptide structure connecting repeats III and IV.

Sodium channel types have been cloned from various tissues. The cloned variants show only small but significant differences in permeation and gating properties. The more striking differences are found in their pharmacological properties. While sodium channels from the central nervous system are potently inhibited by TTX (IC_{50} about 10 nmol L^{-1}), sodium channels from skeletal muscle are less sensitive and cardiac sodium channels are basically insensitive to TTX. These differences are mainly determined by a single amino acid residue in the pore region (rat brain II: F385, rat cardiac I: C374). Some sodium channels from the peripheral nervous systems are also less TTX sensitive. Further cDNA clones, presumably coding for sodium channels of glial and cardiac cells, show quite different structures of the putative voltage sensor and the ion selectivity filter.

The pore-forming α subunits of sodium channels are proteins with molecular mass of about 210 kDa. Although these subunits yield functional channels when expressed in host cells, additional β_1 subunits (~36 kDa) can co-assemble with some of them to modulate the kinetics of activation and inactivation. Furthermore, the rat brain II sodium channel is accompanied by a β_2 subunit (33 kDa) **(Figure 1a)**.

Cellular regulation modifies the function of sodium channels. Phosphorylation of rat brain II sodium channels by cAMP-dependent protein kinase (PKA) leads to a decrease in channel open probability. Phosphorylation by protein kinase C (PKC) reduces the probability of channel opening, but also slows down inactivation. Stimulation of adrenergic receptors in cardiac myocytes can induce an enhanced Ca^{2+} permeability of cardiac sodium channels. Direct interaction with G proteins was shown to influence inactivation and to reduce open probability.

Epithelial sodium channels (ENaC; amiloride-sensitive Na channels) are not gated by voltage and they share no structural similarity with voltage-gated sodium channels. They are composed of three subunits (α, β, γ) each having two transmembrane segments and large extracellular loops. They mediate the reabsorption of sodium in the distal part of the kidney tubules.

Calcium channels

Calcium channels are much more diverse than sodium channels. They have been classified according to their electrophysiological and pharmacological properties. Different nomenclatures are in use; the most common terms the channels T, L, N, P/Q, and R type. Another notation is based on the molecular structures of the α_1-protein subunits (e.g. the cDNA types C, D, and S code for L type channels). Based on their voltage thresholds for activation they are called low-voltage (LVA) or high-voltage activated (HVA) channels. The T type channel activates at low voltages and is thought to be important for the rhythmic activity of some pacemaker cells. P/Q as well as N type channels are involved in the process of neurotransmission; they are in close contact with protein structures in release vesicles. L type channels are expressed in various cell types; they are particularly important for the process of excitation–contraction coupling in muscle cells. In skeletal muscle cells, they are coupled to the ryanodine receptor which mediates Ca^{2+} release from the sarcoplasmic reticulum upon activation of the L type calcium channel.

The strong diversity of calcium channels complicates a classification of their functional properties. While T type channels activate at voltages more positive than about −70 mV, HVA channels activate positive to −20 to −10 mV. Upon depolarization, these channels open within a few milliseconds. T and N type channels undergo rapid inactivation within 20–100 ms, while L type channels inactivate only very slowly. In some cases inactivation is facilitated by Ca^{2+} that enters the cell through the open channel.

Calcium channels are selective for Ca^{2+} and Ba^{2+} over other divalent and monovalent cations. They do not conduct anions. Under physiological conditions, the single-channel conductances range from about 8 pS (T type) to 25 pS (L type). Although the permeability of Ba^{2+} is greater than that of Ca^{2+} in L and N type channels, the permeabilities are about equal in T type channels. The selectivity of Ca^{2+} over monovalent cations vanishes if the concentration of Ca^{2+} is reduced, suggesting that small cations like Na^+ or K^+ can only permeate through the channel when it is not occupied by Ca^{2+} ions. A cluster of four glutamate residues (one from the pore-region of each homologous repeat) in the calcium channel pore forms the

putative Ca^{2+} binding site and, hence, the selectivity filter in the pore.

Pharmacological tools have been used to classify calcium channels. L type channels are modified by dihydropyridines (DHPs; e.g. niphedipine, nitrendipine). While most DHPs inhibit the current flow, the compound BAY K 8644 increases the open probability of the channels. In addition to DHPs, phenylalkylamines (e.g. verapamil) and benzothiazepines (e.g. diltiazem) block L type channels and are used for the treatment of heart diseases and hypertension. The non-DHP-sensitive channels (or non-L-type channels) can be further distinguished by their sensitivities to toxins from the cone snail *Conus* (e.g. ω-Conotoxin GVIA is specific for N type channels) and the funnel-web spider *Agelenopsis aperta* (e.g. ω-Agatoxin IVA is specific for P type channels) as well as to divalent cations (e.g. Ni^{2+} is specific for T type channels). R type channels are not specifically blocked.

As in sodium channels, the pore-forming part of the channel complex is an α_1 subunit of about 170–240 kDa. It associates with a complex of the disulfide-linked subunits δ (27 kDa) and α_2 (125 kDa). Cytosolic β-subunits (58 kDa) modulate channel inactivation. The importance of the γ subunit (25 kDa) is less well understood (**Figure 1a**).

Calcium channels are subject to cellular regulation in numerous ways and only some examples are given here. Calcium channels in cardiac tissue are regulated by adrenergic stimulation. PKA-dependent phosphorylation leads to an increase in channel open probability resulting in a larger Ca^{2+} influx and, hence, in a stronger contraction of the heart muscle. Direct interaction with stimulating G proteins also increases the open probability in heart muscle. N type calcium channels in sympathetic ganglia cells undergo a shift in the voltage dependence of the activation by about +80 mV, which effectively inhibits channel opening, upon interaction with the α subunits of G_o proteins. In addition, PKC-mediated phosphorylation can induce a suppression of the calcium current.

Receptor-operated calcium channels mediate the release of Ca^{2+} from intracellular stores: ryanodine receptors (RyR1, skeletal muscle; RyR2, cardiac muscle; RyR3, brain) and inositol 1,4,5-trisphosphate-activated channels (IP_3R1–4). They share no structural similarity with voltage-gated calcium channels.

Depolarization-activated Potassium Channels

Like sodium and calcium channels, there are many potassium channel types that activate upon membrane depolarization. They are mainly responsible for action potential termination and for setting the resting potential. These depolarization-activated potassium channels (Kv channels) gain their voltage sensitivity from the positively charged S4 transmembrane segment. As indicated in **Figure 3**, these channels are members of the 6TM family. This protein family of voltage-gated potassium channel α subunits is the structural basis for the large functional diversity of potassium channels. While Kv, KQT and Eag channels are 'real' depolarization-activated channels, $K(Ca^{2+})$ and cyclic nucleotide-gated (CNG) channels form additional families with much lower voltage sensitivities but additional regulation by intracellular Ca^{2+} or cyclic nucleotides, respectively (see below). Each of these families divides into smaller subfamilies. The classic Kv channels were first named after mutants of *Drosophila melanogaster* that were used to isolate these clones: *Shaker* (Kv1), *Shab* (Kv2), *Shaw* (Kv3), and *Shal* (Kv4). Depending on the species, these subfamilies have several members that are either individual genes (e.g. Kv1.1 to Kv1.8 from mammals) or splice variants (e.g. *Shaker* variants).

Given the large number of different potassium channel structures, their functional properties are also quite diverse. The activation threshold typically is around −50 to −20 mV (**Figure 2d**), but the speed of activation varies significantly (from milliseconds to several seconds). Speed of inactivation is a good criterion for the discrimination of potassium channels. A type channels inactivate within several milliseconds (**Figure 2c**), while delayed rectifier channels do not undergo inactivation even if the depolarization lasts several seconds (**Figure 2c**). There are many channel types ranging between these extremes. The activation mechanism is associated with a movement of the S4 voltage sensor, which finally triggers a conformational change leading to the opening of the K^+-selective pore (**Figure 2a**). There are at least two molecular mechanisms leading to channel inactivation. The process of N type inactivation involves the N-terminal structure of the polypeptide. About 20–30 terminal amino acids form an inactivating structure that can occlude the pore from the cytosol, leading to inactivation (Hoshi *et al.*, 1990). As the inactivating domain is tethered to the rest of the membrane-based protein structure via a putatively flexible chain of amino acid residues, this mechanism is often referred to as 'ball-and-chain mechanism', as previously postulated for the inactivation of sodium channels. Only a few potassium channel α subunits exhibit N-terminal structures that can lead to N-type inactivation (e.g. *Shaker*, rKv1.4, rKv3.4). The structures of some of these 'ball' peptides have been recently determined via NMR methods. Other channel types close by a mechanism called C type inactivation. The molecular mechanisms involved are not well understood; the pore structure and the occupancy by permeating K^+ ions seems to be of particular importance. Some potassium channels (e.g. rKv1.4, h-erg) are very sensitive to extracellular K^+ – these channels close when $[K^+]_o$ drops markedly below physiological levels.

Potassium channels are the most selective ion channels as some of them display a permeability ratio for K^+ over

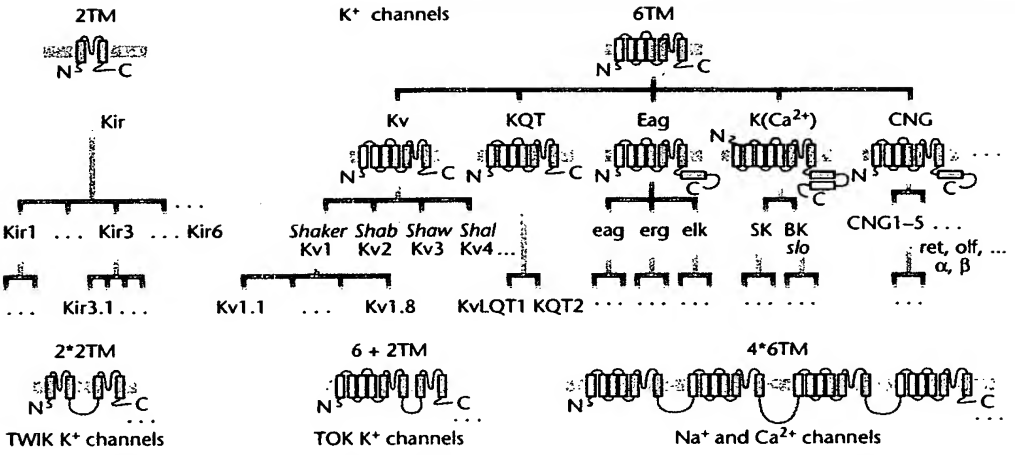

Figure 3 Overview of the gene family of potassium channel-forming protein subunits and related proteins, showing the most important members only (modified after Wei *et al.*, 1996). Based on potassium channels with 2 and 6 transmembrane segments (2TM and 6TM), further channel-forming proteins have presumably evolved by gene duplication and splicing: 2*2TM (TWIK channels), 6+2TM (TOK channels), and 4*6TM (sodium and calcium channels). Several members of the 2TM and 6TM families are indicated. Owing to the rapid progress in the cloning of channel genes, there are many more members expected to be isolated, as indicated by dots.

Na^+ of > 100. They are virtually impermeable for divalent cations or anions. The single-channel conductances of potassium channels span a wide range from a few picosiemens ($SK(Ca^{2+})$) to more than 100 pS ($BK(Ca^{2+})$). Common to all K^+-selective channels is the so-called signature sequence Gly-Tyr-Gly in the pore domain (in some cases Gly-Phe-Gly). Modification of this motif reduces ion selectivity. Deletion of Tyr-Gly yields ion channels with permeation characteristics similar to those of CNG channels (Heginbotham *et al.*, 1992; see below). The crystal structure of a potassium channel from *Streptomyces lividans* (KscA) has been determined and suggests that the carbonyl moieties of the GYG-polypeptide backbone interact with the K^+ ions to form a selectivity filter (Doyle *et al.*, 1998). The pore diameter is about 0.4 nm at the narrowest part.

Pharmacological tools proved very useful in characterizing various potassium channel types prior to molecular cloning. Quaternary ammonium ions, in particular tetraethylammonium (TEA), have been widely used to characterize potassium channels. They reduce the K^+ current by blocking the open potassium channel pore when applied from the external or internal side. Mutagenesis of the pore region in *Shaker* potassium channels revealed interaction sites with external (D431, T449) and internal (T441) TEA. Aminopyridines, in particular 4-aminopyridine (4-AP), block some potassium channels by interacting with the pore region from the cytosolic side. More specific are peptide toxins from the venoms of various animals: some voltage-gated potassium channels are particularly blocked by dendrotoxins (green mamba snake *Dendroaspis augusticeps*), charybdotoxin (scorpion *Leiurus quinquestriatus*), and noxiustoxin (scorpion *Centruroides noxius*). In addition, many potassium channels are blocked by Cs^+ and Ba^{2+} ions.

Potassium channels are expressed in almost all animal cells and they are also found in plant cells, suggesting a long phylogenetic history. The rapidly activating and inactivating channels are typically found in excitable cells such as neurons or muscle cells.

Kv channels have a 4-fold symmetry as four α subunits (e.g. *Shaker* ~70 kDa) form a pore-forming channel complex. Assembly of these subunits is determined by specific assembly sites such that channels can be formed as heterooligomers (typically within one subfamily). Auxiliary β subunits for voltage-gated potassium channels (Kvβ subunits) are cytosolic proteins (28–47 kDa) that assemble with the α subunits at the N-terminal domain (**Figure 2a**). While some of these Kvβ subunits (e.g. Kvβ1.1) induce fast inactivation in delayed rectifier channels (Rettig *et al.*, 1994), the functional role of others (e.g. Kvβ2) is not yet clear. Like Kvβ4 (28 kDa) it may function as a chaperone. The small protein IsK (also called minK) has one putative transmembrane segment and modifies the function of voltage-gated potassium channels (e.g. KvLQT1) upon co-assembly.

Potassium channels are subject to cellular regulation in various respects. By specific modification of residues in the N-terminal structure, the rapid inactivation of A type channels can be modulated (e.g. phosphorylation of Ser and Thr residues, redox regulation of Cys, oxidation of Met). In addition, Ser/Thr- and Tyr-phosphorylation was shown to inhibit potassium channels in various preparations; a classic example is the PKA-mediated phosphorylation of the as yet not molecularly characterized Ks channels in the presynaptic nerve ending of the snail

Aplysia californica, which constitutes a mechanism for synaptic potentiation.

Ca^{2+}-dependent potassium channels

A subfamily of 6TM potassium channels is activated by intracellular Ca^{2+} ions. Based on their single-channel conductance, these channels are classified as large-conductance ($BK(Ca^{2+})$, also called Maxi-K channels, 100–300 pS) and small-conductance ($SK(Ca^{2+})$, 4–14 pS) channels. While $SK(Ca^{2+})$ channels are activated at rather low intracellular Ca^{2+} concentrations (10–100 nmol L^{-1}), more Ca^{2+} is needed to activate $BK(Ca^{2+})$ channels (1–10 μmol L^{-1}). In addition, activation of $BK(Ca^{2+})$ channels depends on the membrane potential, facilitating channel opening at depolarized voltages. $K(Ca^{2+})$ channels constitute an important link between intracellular Ca^{2+} and cell excitability. They therefore play a major role in the regulation of electrical activity such as spike frequency adaptation and electrical pattern generation in pacemaking neurons, generation of afterhyperpolarizations in central neurons, transmitter release, and regulation of vascular and visceral smooth muscle contractility.

$K(Ca^{2+})$ channels are effectively blocked by peptide toxins: $BK(Ca^{2+})$ is blocked by charybdotoxin and iberiotoxin (scorpion *Buthus tamulus*), $SK(Ca^{2+})$ is blocked by apamin, which is obtained from bee venom.

$K(Ca^{2+})$ channels have been cloned from the *slowpoke* mutant of *Drosophila melanogaster*; homologous clones from various tissues code for $BK(Ca^{2+})$ channels (e.g. h*slo*). The Ca^{2+}-sensing domain of $K(Ca^{2+})$ channels resides in the C-terminal end of the polypeptide. In contrast to Kv channels, the N-terminal domain of $BK(Ca^{2+})$ channels seems to be folded so that there exists an additional putative transmembrane segment (S0), resulting in an extracellular amino-terminus. An auxiliary β subunit of $K(Ca^{2+})$ channels is not related to Kvβ subunits; it has a molecular mass of ~30 kDa, two putative transmembrane segments and a large extracellular loop. It modifies the Ca^{2+} and voltage sensitivity of $BK(Ca^{2+})$ channels upon co-assembly with the S0 segment, resulting in a much larger open probability at resting voltages.

Cyclic Nucleotide-gated Channels

Cyclic nucleotide-gated (CNG) channels play a major role in the signal-transduction pathways of vertebrate photoreceptor cells and olfactory neurons. In photoreceptor cells, light indirectly induces the breakdown of cGMP (cyclic guanosine monophosphate). The decrease in $[cGMP]_i$ leads to the closing of cGMP-gated channels and a hyperpolarization of the photoreceptor cell as these channels conduct Na^+ and Ca^{2+}. By means of 7-helix-receptors and the activation of G proteins, odorants increase the level of intracellular cAMP (cyclic adenosine monophosphate), which activates cAMP-gated channels, leading to depolarization of the olfactory neurons.

CNG channels belong to the family of 6TM channels; however, they retain only a very weak voltage dependence. The pore domain shares similarities with those of K^+-selective channels but lacks the signature sequence Gly-Tyr-Gly. The channels are therefore rather unselective among the monovalent cations. In addition, a significant amount of Ca^{2+} permeability gives them a particular physiological role. A part of the C-terminal domain harbours a cyclic nucleotide-binding motif similar to those found in cGMP- and cAMP-dependent kinases. Retinal and olfactory CNG channels share about 60% sequence homology. While retinal channels respond selectively to cGMP, olfactory channels are activated by cGMP and cAMP at about equal concentrations. The interaction with Ca^{2+}/calmodulin (Ca^{2+}/CaM) reduces the sensitivity of CNG channels for cyclic nucleotides. For olfactory CNG channels in particular, which have a Ca^{2+}/CaM binding site in the N-terminal domain of the α subunit, this mechanism provides a means for odorant adaptation.

CNG channel α subunits co-assemble with β subunits (also called α_2 subunits), which by themselves do not form functional channels, in a putative $\alpha_2\beta_2$ stoichiometry. The membrane-based portion of these proteins is similar to the α subunits (6TM motif). The C-terminal domain of the β subunit from bovine rod CNG channels comprises, besides the binding site for cyclic nucleotides, a binding domain for Ca^{2+}/CaM. The N-terminal domain harbours a large GARP (glutamic acid-rich protein) structure.

Inward Rectifying Channels

Inward rectifying potassium channels (Kir channels) stabilize the resting membrane potential as they are active at hyperpolarized voltages. The channels close upon depolarization of the membrane and have previously been termed anomalous rectifiers. A subclass of Kir channels requires additional activation by G proteins.

The voltage-dependent activation mechanism of Kir channels can be attributed to channel block by intracellular polyvalent cations (Mg^{2+} and polyamines). While the channels are not blocked at hyperpolarized voltages, depolarization drives the blocking ions into the channel pore. Kir channels are selective for K^+ over Na^+ similarly to Kv channels and they share a homologous pore domain.

Kir channels are formed from tetramers of 2TM subunits (**Figure 2**). As indicated in **Figure 3**, there are several subfamilies (Kir1–Kir6) cloned from various tissues and coding for channel-forming protein subunits of diverse size (e.g. mKir2.1 ~48 kDa, Kubo *et al.*, 1993). Kir1 channels show weak and Kir2 channels show strong

inward rectification. The members of the Kir3 subfamily are gated by direct interaction with the βγ subunits of GTP-binding proteins. Therefore, Kir3 channels constitute the functional part of the K(ACh) channels in cells of the sinoatrial node and the atrium: acetylcholine (ACh) activates a muscarinic 7-helix receptor; this activates G proteins that finally cause an opening of Kir3 channels. Apparently ACh-activated Kir channels in the heart occur as heterotetramers (e.g. Kir3.1 + Kir3.4). Kir6 proteins are the functional part of K(ATP) channels as expressed in β-cells of the pancreas. These channels close in response to an increase in intracellular ATP. The channel protein couples to a sulfonylurea receptor, a member of the ATP-binding protein family (ABC proteins), and thereby becomes indirectly the target of clinically used potassium channel openers such as diazoxide or cromacalim. Anti-diabetic drugs such as glibenclamide or tolbutamide act antagonistically as they block K(ATP) channels by binding to the sulfonylurea receptor.

Kir channels can be downregulated by PKA- and PKC-mediated phosphorylation. And at least for some members of the Kir family, a coupling to cytoskeletal proteins seems to be important. Cs^+ and Ba^{2+} ions are often used to block these channels.

Inward rectification is not only mediated by Kir channels. KAT channels, cloned from plant cells, are members of the 6TM family but also give rise to inwardly rectifying current. H-erg channels that are expressed in cardiac cells and several cancer cell lines obtain certain inwardly rectifying characteristics by a combination of rapid inactivation and slow channel deactivation. This results in only minimal outward current during depolarization but marked inward current during the subsequent hyperpolarization.

In addition to the 2TM and 6TM motifs of pore-forming protein subunits there are variants with 2TM + 2TM (TWIK channels; abundantly expressed) as well as with 6TM + 2TM (TOK channels; thus far found only in yeast) motifs giving rise to voltage-dependent K^+ currents (**Figure 3**).

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